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14. ABSTRACT

Purpose: We are evaluating efficacy of GM6001, a matrix metalloproteinase (MMP) inhibitor in a murine model of spinal cord injury (UCSF) and in dogs (Texas A & M, TAMU) that sustain naturally occurring spinal cord injuries resulting from spontaneous intervertebral disk herniation.

Scope: These studies have focused on efficacy of GM6001 in the context of optimal therapeutic window and dependency on injury severity, using clinically relevant outcome measures that include neurologic assessments and assays of bladder function.

Major findings:

-Developed reproducible models of graded spinal cord injuries in the mouse.
-Spinal cord injury in mice resulted in marked injury severity-dependent changes in bladder function including the emergence of uninhibited bladder contractions, increased bladder volume/weight, and increased urinary retention.
-Conducted the first blinded, randomized study to assess efficacy of GM6001 when delivered 8 hours after a moderate spinal cord injury in mice. Though group sizes were small, these data show promising benefit of GM6001 in terms of neurologic improvement and decreased abnormal bladder contractility relative to vehicle controls.
-Completed pharmacokinetic study of GM6001 in 10 dogs. The study supports the rapid development of maximal plasma concentration after subcutaneous delivery, the presence of plasma drug levels capable of inhibiting MMPs *in vitro*, and the short-term safety of the drug. Plasma drug levels following a single dose are sustained at a significant level likely to result in MMP inhibition for approximately 72 hours, which support a single dose strategy in the clinical trial.
-Completed normal dog cystometry in 10 dogs
-Enrolled 6 dogs with acute intervertebral disk herniation-associated spinal cord injury into serial cystometry study. As expected, dogs with spinal cord injury that are non-ambulatory lack normal voiding reflex, have larger bladder capacity, have elevated post-cystometry baseline pressure, and have larger residual volume compared to measures taken during recovery.

Significance: Preliminary data demonstrate that GM001 improves long-term neurologic outcome and reduces abnormal bladder function in a murine model of spinal cord injury when treatment is delayed for 8 hours after injury. Such a delay is relevant to the theater, where initiation of therapy may not be feasible within the first few hours post injury. These findings serve as a foundation for the dog studies. To date, we have studied the pharmacokinetics of GM6001 in the dog, a key step in determining timing and dosing of the drug after spinal cord injury. We have also developed the necessary tools to assess bladder function after spinal cord injury in dogs. Thus, we are now in an optimal position to not only further verify our initial promising findings of GM6001 in the spinal cord injured mouse but to also translate this effort to a more naturally occurring spinal cord injury in dogs. Validation of GM6001 in two species would be a power argument for advancing this drug to human clinical trials.

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INTRODUCTION **1**

The primary objectives of this research are to evaluate the efficacy of a general inhibitor of matrix metalloproteinases, GM6001, in both a mouse model of spinal cord injury and in dogs who have sustained a naturally occurring spinal cord injury resulting from the sudden rupture of an intervertebral disk. The study builds upon our earlier work in the murine model of spinal cord injury, which showed that GM6001 significantly improved neurologic outcome when given 3 hours post injury after a moderate spinal cord injury (1). Thus, the goal here is to determine if GM6001 is likewise efficacious if the window of therapeutic intervention is extended and if the injury is more severe. In addition, we will determine if GM6001 improves bladder function. Findings from the mouse studies serve to inform the dog preclinical trial, where the focus will be on initial categorization of dogs according to severity of injury and assessment of GM6001 efficacy as determined by both neurologic and urologic assessments.

Please note that each task is indicated in bold. We also request several changes to our tasks related to Specific Aim 1. These requested changes are indicated in bold italics.

BODY
UCSF Site:

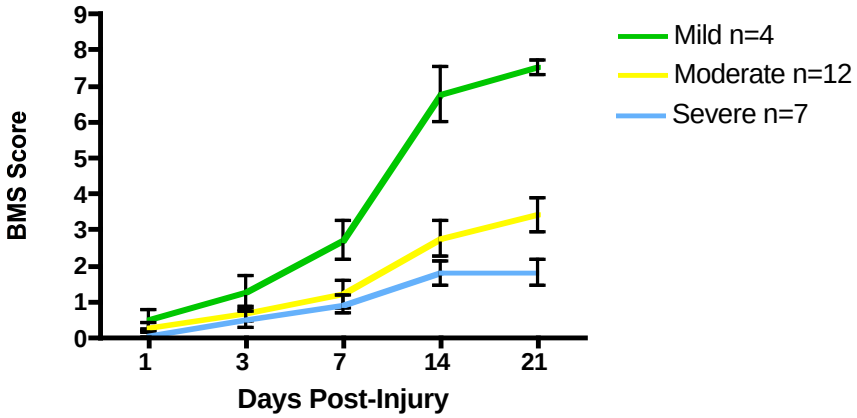
Specific Aim 1
Task 1. Refine the therapeutic window for GM6001 in mice
1a. Obtain animal use protocol approval to study 165 mice (months 1-4)

We received approval from the UCSF IACUC and ACURO to conduct these studies.

1b. Compare neurologic recovery in 30 mice when GM6001 is initiated at 8 hours post injury. (months 5-6)

For reasons described below (Texas A & M, Specific Aim 2, Task 1b) GM6001 was not available until month 8th of this project. In the interim, we refined our injury model so that we could reproducibly generate both moderate and severe spinal cord injuries (as required for Specific Aim 1, Task 2a) and defined a series of abnormal urologic parameters that reflect spinal cord injury including uninhibited bladder contractions, peak bladder pressure, bladder volume, and bladder weight. These experiments provide a foundation for Specific Aim 1,Task 3A. Finally, beginning in month 10, we began Task 1b. Below summarizes our findings.

To *confirm a reproducible, graded model of spinal cord injury,* male, C57BL/6 mice were subjected to a 2 gm weight dropped 5 cm (mild injury), a 2 gm weight dropped 7.5 cm (moderate injury), or a 3 gm weight dropped 5 cm (severe injury) onto the cord exposed at the T 9 vertebral level (Figure 1). Severity was defined based upon the BMS scale where a score of 0 indicates hindlimb paralysis and a score of 9 reflects normal hindlimb locomotor function. The more mildly injured animals showed scores of about 7.5 (frequent to consistent stepping and mostly coordinated in their locomotion). The moderately injured group scored about 3.5 (occasional plantar stepping). The severely injured animals scored about a 2 (hindlimb movement is limited to extensive ankle movement).



Two way ANOVA

Interaction	p<0.0001
Time	p<0.0001
Injury Severity	p=0.0002

Figure 1. Production of a graded reproducible model of spinal cord injury in the mouse.

We next evaluated urologic status at approximately 35 days after spinal cord injury, focusing on 4 measures- namely uninhibited bladder contractions, residual urine, bladder weight, and bladder volume.

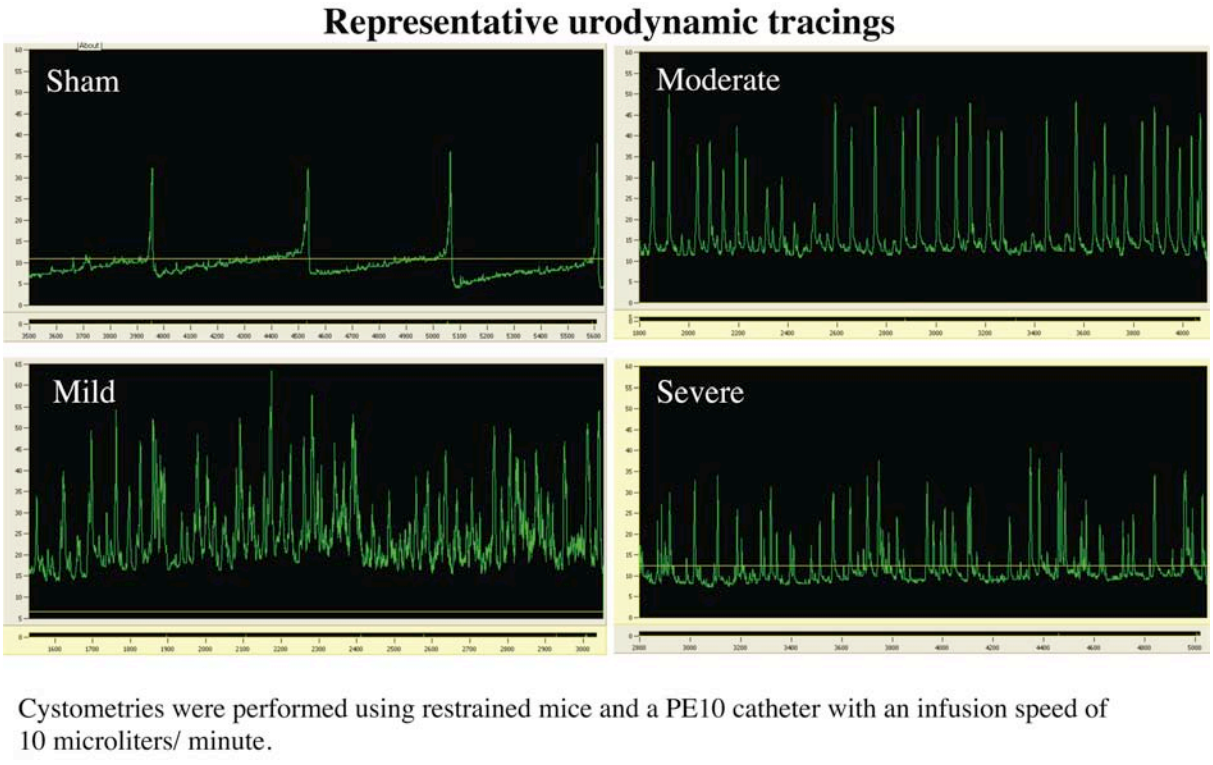


Figure 2. Uninhibited bladder contractions after mild, moderate, or severe spinal cord injury. Note the relative abundance of these contractions after mild injury relative to the more severely injured spinal cord.

Representative urodynamic tracings (Figure 2), resulting from cystometry in mice subjected to mild, moderate or severe spinal cord injury, revealed distinct differences between mild and severe injury with the former showing much more prominent uninhibited bladder contractions relative to the latter. We next compared uninhibited bladder contractions after mild and moderate spinal cord injury (Figure 3, Left Panel). Based upon the Kruskal–Wallis test followed by Dunn's Multiple Comparison Test, there were significantly increased numbers of bladder contractions after mild or moderate injury relative to the sham control group. While

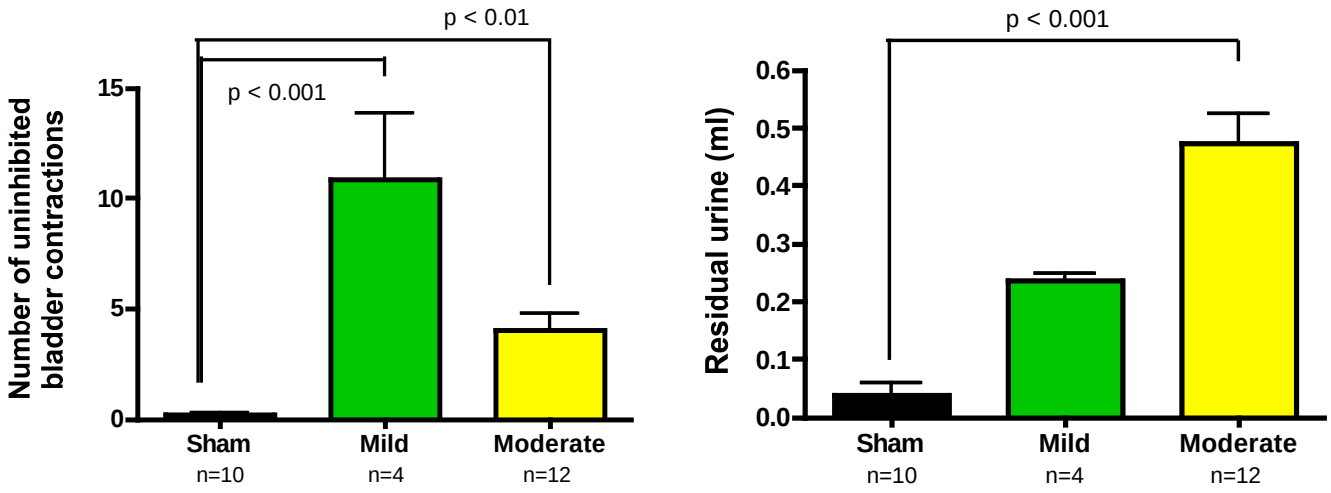


Figure 3. Left Panel: Quantification of uninhibited bladder contractions after a mild and moderate spinal cord injury. Right Panel: Quantification of residual urine.

qualitatively the mild group appeared to have greater number of contractions that the moderate group, these findings were not significant. Residual urine (Figure 3, Right Panel) was significantly greater in the more moderately injured group (Kruskal-Wallis followed by Dunn's Multiple Comparison Test). In contrast, peak voiding pressure showed no differences between injury and sham groups (Figure 4). Finally, we analyzed bladder volumes (Figure 5) and bladder weights (Figure 6) using the same statistical approaches. While bladder weight increased incrementally after mild and moderate injury, bladder volume remained unchanged, relative to shams, after a mild injury, whereas significantly increased after a moderate injury.

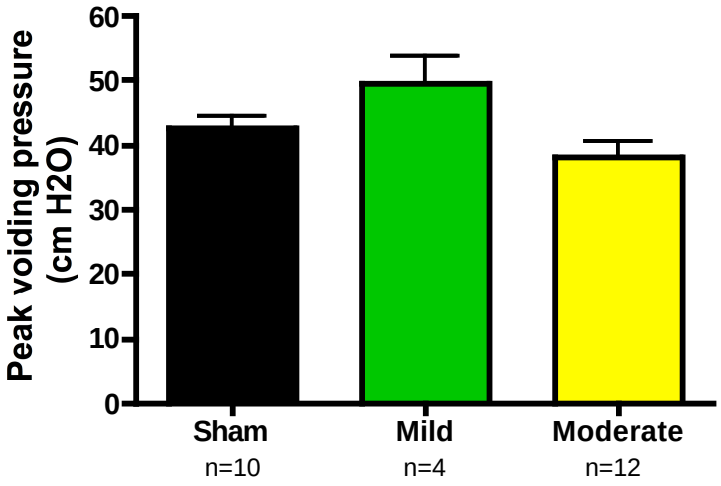


Figure 4. Peak pressure revealed no differences between groups.

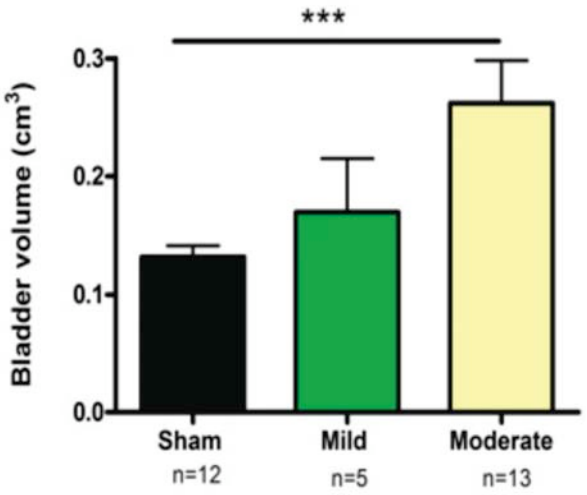


Figure 5. Bladder volume significantly increased after a moderate injury whereas there are no differences between mild and sham controls. ***P<0.001,

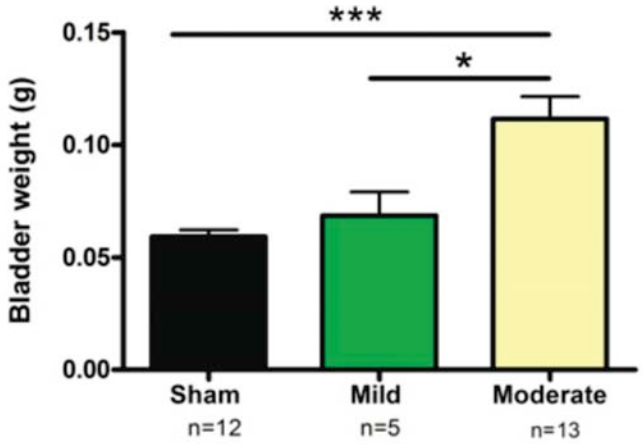


Figure 6. Bladder weight significantly increases after either mild or moderate injury compared to sham controls. ***P<0.001, **P<0.01

In summary, we show that we can generate reproducible graded levels of injury severity and that urologic status shows injury severity-dependent changes with uninhibited bladder contractions most pronounced after a more mild injury and residual urine, bladder volume and bladder weight all key features of the moderate level of injury. We believe that reduced uninhibited bladder contractions with greater severity of spinal cord injury may reflect prolonged over distension of the bladder wall, which may either damage the muscle layer or result in aberrant remodeling such that the muscle wall has reduced capability of contracting.

Finally, we evaluated the efficacy of GM6001 when give 8 hours after injury. A total of 25 C57Bl/6 adult male mice were subjected to a moderate contusion injury at T9. These mice were then randomly selected for drug (N= 12) or vehicle (n= 13). At 8 hours post injury, all mice were evaluated using the BMS scale. Exclusion criteria were as follows: any animal showing an average score of > 0.5 at 8 hours post-injury or

morbidity as defined in UCSF IACUC and ACURO approved protocols. GM6001, dissolved in 4% carboxy methyl cellulose at a concentration of 20mg/ml, was given intraperitoneally at 100mg/kg at 8 hours post-injury followed by repeated administration every 12 hours for 3 days. The vehicle control group received the same timing/route of administration. Of the 12 GM6001 treated mice, a total of 5 were excluded from the study due to early death or early morbidity. Of 13 mice that were treated with vehicle, 4 were removed from study due to morbidity, 2 met exclusion criteria at 8 hours post injury, and 2 others had injury device malfunction. Thus, neurologic recovery was evaluated in N= 5 for the vehicle and N= 7 for drug.

Two-way repeated measures ANOVA of BMS score revealed the following: P= 0.58 for interaction, P<0.0001 for time, and P= 0.16 for treatment (Figure 7). That is, all animals showed significant improvement in locomotor ability over time. However, there was no difference between vehicle and drug treated groups.

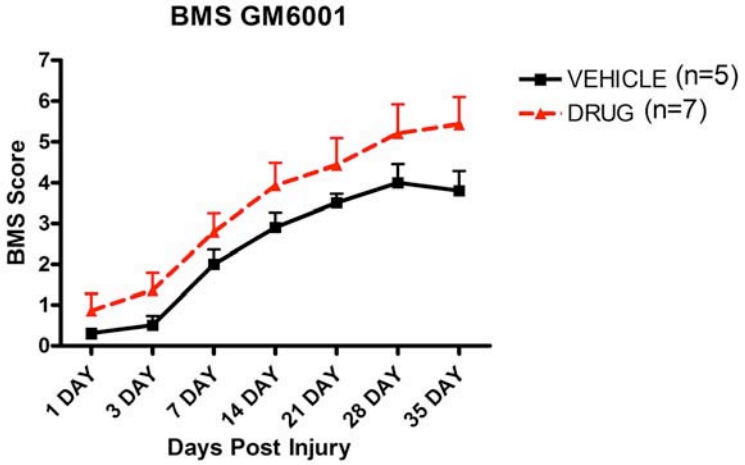


Figure 7. Male C57bl6 mice were subjected to a moderate spinal cord injury and treated with either GM6001 or vehicle beginning 8 hours post injury and every 12 hours thereafter for 3 days. While both groups improved with time, there were no differences between drug and vehicle, based upon the BMS scale.

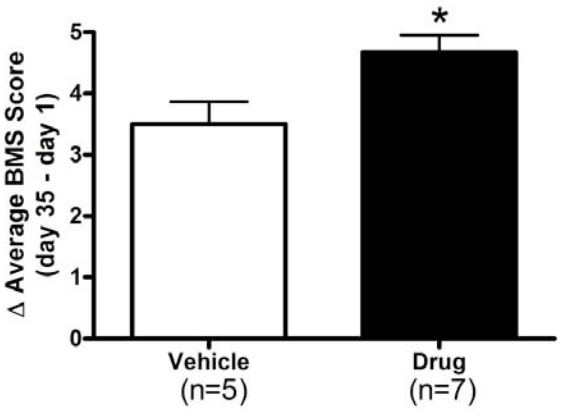


Figure 8. Comparison of initial BMS scores at 1 day versus 35 days, revealed a significant improvement in the GM6001-treated group.
* P=0.025

We next evaluated improvement between the groups by comparing initial BMS scores at day 1 relative to final BMS scores at day 35 (Figure 8). Based upon a Student T-test, the drug treated group shower greater improvement that the vehicle group (P= 0.025). Finally, since weight supported stepping is considered to be a very favorable outcome, we evaluated the percentage of mice that showed frequent stepping (Figure 9).

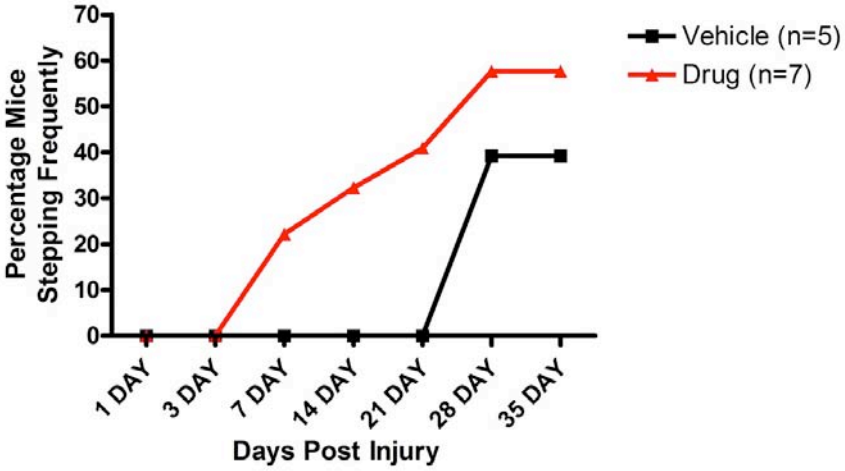


Figure 9. Evaluation of percentage of mice, subjected to a moderate spinal cord injury, that shows hindlimb stepping over time

Statistical comparisons (2-way ANOVA) were done on percentages that were transformed into arcsin values using the formula ASIN(SQRT(A2/100))*180/PI . Approximately 60% of mice, treated with GM6001, showed frequent stepping whereas only 40% achieved that degree of recovery in the vehicle treated group. Based upon 2-way ANOVA there was a significant effect of both treatment and time (P= 0.017) and P= 0.015).

Finally, we have analyzed a cohort of bladders from these animals by cystometry. While we saw no differences in bladder volume, bladder weight or residual urine, the GM6001 treated group showed a significant

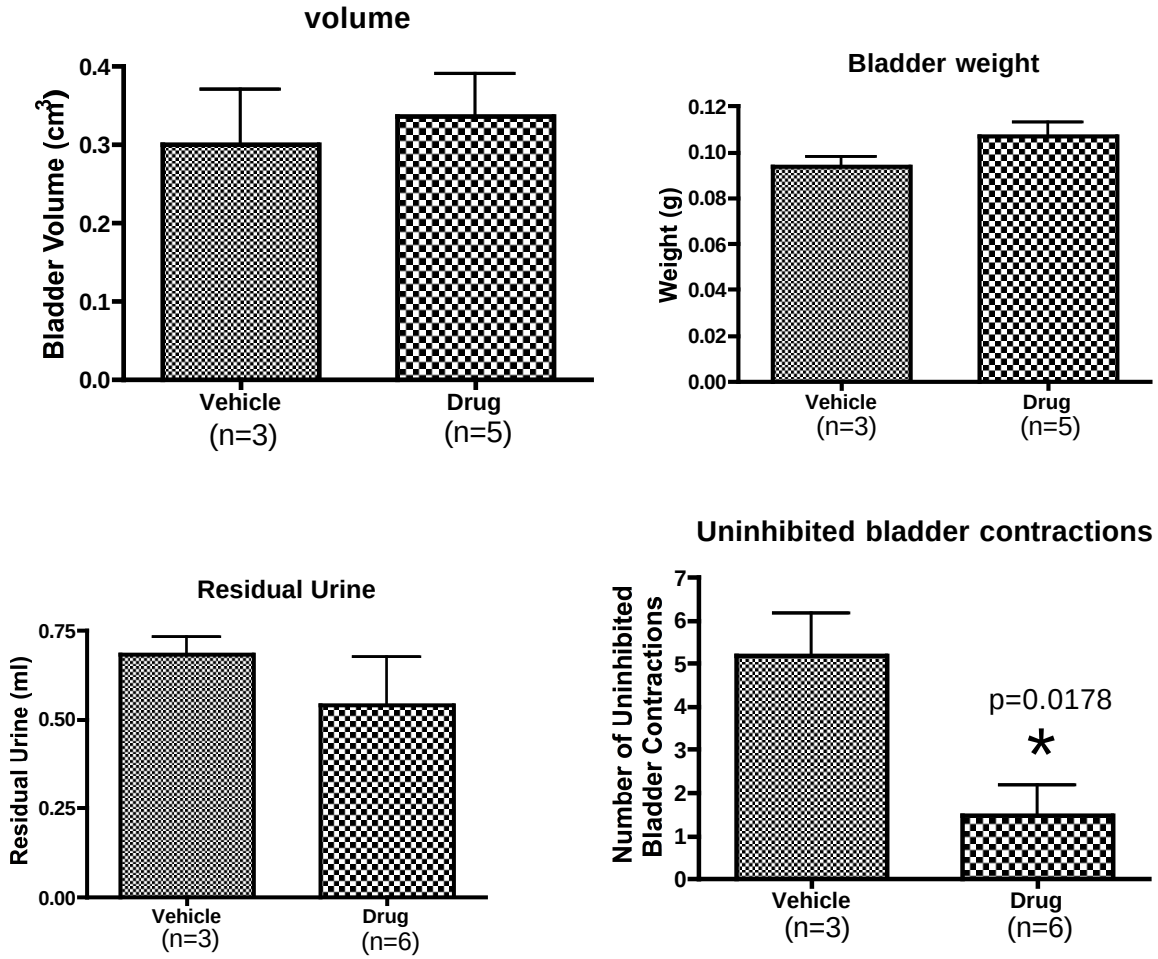


Figure 10. Analysis of bladder function after a moderate spinal cord injury in the mouse. Cystometry was conducted in those animals that were also evaluated for neurologic function (Refer to Figure 7-9). There were signature reductions in number of uninhibited bladder contractions and peak pressure (Unpaired Students T-test.

reduction in uninhibited bladder contractions, one of the key features of dyssynergia (Figure 10).

In summary, this initial study to assess the efficacy of GM6001 shows promising results, despite the fact that animal numbers per group were relatively low. Importantly, beneficial effects based upon neurologic assays correspond to reductions in abnormal bladder contractility (uninhibited bladder contractions). Together, we are excited about the potential for GM6001 in not only improving neurologic function but also reducing urologic dysfunction.

Based upon autopsy of mice that died or were euthanized acutely after injury due to morbidity, we believe that repeat intraperitoneal injections caused intraabdominal local tissue damage. (Deaths and morbidity occurred within the first 4 days after injury). ***To address this issue, we propose a change to our experimental design, whereby we administer GM6001 or vehicle (DMSO) subcutaneously using the same dose and timing of administration. This route and vehicle are what will be used in the dog study at TAMU, and as such would better parallel that study. We have submitted a revision to our UCSF IACUC and once approved we will then forward on to ACURO for review. Once these are approved, we will request permission from the Grants Office's Representative to eliminate task 1c, so that we can repeat task 1b, using the subcutaneous route and DMSO as the vehicle. The value of repeating this study is that we have opportunity to confirm our initial positive findings in a larger group of animals (n= 15/group) that are treated in a similar fashion to what is planned for the preclinical trial in dogs.*** If approved the time line for completion would be months 13-14.

1c. Compare neurologic recovery in 30 mice when GM6001 is initiated at 6 or 12 hours depending on the results 1b. (months 7-8)

We will seek permission from the Grants Office's Representative to eliminate task 1c, so that we can repeat task 1b, testing a subcutaneous route of administration.

Task 2. Determine if GM6001 will be efficacious after a more severe SCI in mice.

2a. Compare neurologic recovery in 30 mice after a severe SCI. (months 9-10)

We anticipate a delay with a plan of completion in months 16-17.

Task 3. Determine if GM6001, when optimally delivered, will improve bladder function in mice.

3a. Compare urologic function in 30 spinal cord injured mice treated with either vehicle or GM6001. (months 11-13)

If we find that GM6001, given subcutaneously, at 8 hours post injury results in improved neurological outcome, we will use these same animals to study bladder function. This would be completed in months 13 and 14**. *If we find that GM6001 fails to improve outcome, we will seek permission from Grants Office's Representative to evaluate bladder function in mice that have been given GM6001 3 hours post injury, a time point, which we have previously shown results in a significant improvement in locomotor function*** (1).

Task 4. Analysis of lesion epicenter and serotonergic fiber tracks caudal to a SCI in mice.

4a. Perfuse a maximum of 165 animals with fixative, remove the cords, and stain with luxol fast blue or immunostain for serotonergic fiber tracks. (months 5-24)

All animals thus far studied have been perfused with fixative, cryoprotected, and frozen.

4b. Quantify residual white matter and serotonergic fiber tracks caudal to the injury in a maximum of 165 animals. (months 12-32)

We will begin these histologic assessment in month 13.

4c. Statistically analyze data. (months 30-36).

Our plan is to analyze data at the completion of each task. Thus, we have analyzed the first GM6001 study in month 11 and expect to complete all of the behavioral assessments by month 20. CSF samples from dogs will be analyzed in months 30-35.

Specific Aims 2-3

Task 4. Measure MMPs in CSF in dogs

4a. Collect serum from dogs, conduct fluorogenic assays, and analyze data in approximately 125 dogs. (months 12-30)

Our group has recently entered into a collaboration with Dr. Michael Heller at UC San Diego. Through that work, we have now been able to demonstrate that GM 6001 has *in vitro* activity against MMP-2 and MMP-9 at concentrations far below those achieved in dog plasma (40-80 ng/mL) and results in near maximal inhibition of metalloproteinases. These data together with the pharmacokinetics support the relevance of this strategy and also suggest that single dosing is likely adequate to achieve reasonably sustained MMP inhibition in dogs. Moreover, this novel assay will serve as a complementary approach to work at UCSF to address MMP activity in CSF using fluorogenic assays.

BODY

Texas A & M Site:

On 11/6/11 a sub-award agreement between UCSF and Texas A&M University (TAMU) was reached, permitting the ordering of materials to begin work at TAMU. Approvals for key purchases including urodynamics equipment (Laborie Goby), study drug (GM 6001, SAI Advantium, India), and pharmacokinetic analysis (KCAS LLC, Kansas, USA) were obtained by mid-December 2011.

Specific Aim 2

Task 1. Study of pharmacokinetics of GM 6001 in 10 purpose bred dogs (months 1-12)

1a. Obtain animal use protocol approval at Texas A&M University (months 1-4)

Animal use protocols were approved at TAMU on 8/12/11

1b. Order GM 6001 drug (months 1-4)

We were able to obtain permission through our Office of Sponsored Research (OSR) to order GM-6001 in mid-December 2011. A contract was executed with SAI Advantium and processing of the drug began in early January 2012. On March 22 2012, production of 110 g of GM-6001 at HPLC > 98% was completed. The drug was received at TAMU on 4/7/12. Unfortunately, delays associated with obtaining a sub-contract agreement, executing a contract with SAI, and actual drug production resulted in GM 6001 being available in month 8 of the study as opposed to the planned month 4.

1c. Order 10 purpose bred dogs (month 4)

Beagle-like dogs were obtained through the TAMU comparative medicine program in late April 2012, following the availability of GM-6001. Dog purchase was delayed as a result of the delays in obtaining GM 6001.

1d. Receive purpose bred dogs, allow for acclimatization (month 5)

Dogs were received and acclimatized by early May 2012.

1e. Perform physical examination and obtain complete blood count, chemistry, and urinalysis (month 5.5-6)

1f. Anesthetize dogs, place jugular catheters, and deliver GM 6001 as a single 100 mg/kg subcutaneous dose (5 dogs) and two 100 mg/kg doses separated by 12 hours (5 dogs) (month 5.5-6)

(Figure 1, In Supporting Data)

1g. Serial serum acquisition (month 5.5-6)

Objectives 1e-1g were accomplished in mid-May 2012.

1h. Samples stored at -80C and shipped to PharmCats for gas chromatography (month 6)

KCAS was selected as an alternative vendor for pharmacologic studies as they had a lower bid than PharmCats and more rapid turn-around. Samples were shipped to KCAS in mid-May 2012.

1i. Samples processed by gas chromatography at PharmCats (months 6-10)

By mid-June 2012, KCAS generated pharmacokinetic data from dogs. These data were available within the anticipated time frame.

1j. Dr. Fajt to analyze pharmacokinetic data (months 10-12). Dr. Fajt will calculate drug elimination half life, peak drug concentration, time to peak concentration, area under the curve, and absorption half life. If serum levels remain elevated beyond the target duration of <5 in the single dose group, drug dose in the IVDH study population will be appropriately adjusted. If serum GM 6001 levels are not present for at least 3 days with a single dose protocol, we will consider a 2 dose paradigm in the IVDH study population.

Dr. Fajt received pharmacokinetic data in mid-June 2012 and completed her analysis by July 1st 2012, 2 months ahead of the SOW schedule.

Summary Task 1: Delivery of GM-6001 was accomplished in 10 purpose bred dogs. All dogs were clinically normal prior to drug administration based on physical examination, neurological examination, complete blood count, serum biochemistry, urinalysis, and CSF analysis. There were few adverse events associated with drug delivery: 10/10 dogs exhibited mild regional hyperesthesia at the delivery site which abated within 1-3 minutes and 10/10 dogs developed transient swelling at the delivery site. Swelling at the delivery site was 2-5 cm in diameter and at the time of the conclusion of the study had decreased in size to 1-3 cm. We have recognized

similar swellings in a 4 dog safety study of GM 6001 our group previously completed and in 35 dogs that have been administered the drug at 100 mg/kg S.C.

Preliminary analysis of the pharmacokinetics of GM 6001 delivered S.C. in dogs suggests a rapid absorption and initial elimination followed by long-elimination half-life ("flip-flop phenomenon"). This pattern required a non-compartmental analysis. GM-6001 was detected in plasma at the earliest time point following delivery (5
minutes) and had a mean time to maximal concentration (Tmax) of 0.7 hours (S.D. +/- 1.3 hours). The mean maximal concentration (Cmax) was 1370 ng/mL (S.D. +/- 361 ng/mL). The calculated elimination half-life for a single dose is 524 hours (S.D. +/- 428 hours). The mean concentration of GM-6001 following single dose delivery was 80 ng/mL (S.D. +/- 20 ng/mL) at 96 hours.

Task 2. Compare motor recovery in dogs with IVDH (intervertebral disk herniation) associated SCI that receive saline placebo, DMSO vehicle, or GM 6001 (months 1-36)

2a. Obtain animal use protocol approval at Texas A&M University (months 1-4)

Animal use protocols were approved at TAMU on 8/12/11

2b. Obtain Clinical Research Review Committee approval (months 1-4)

Clinical Research Review Committee approval was granted at TAMU on 8/12/11

2c. Advertise clinical study via electronic brochures (months 6-18)

In February 2012 UCSF and TAMU began efforts to announce the study to media in order to develop interest in the general public. Stories were featured in the NY Times, ABC News, MS NBC, and on the Today Show website describing this unique collaboration. On June 1 2012 TAMU began efforts to advertise the study to our referring veterinarian population. We have developed 2 electronic brochures through our media resource department. We have announced that enrollment has opened for Specific Aim 3 Task 1 through a listserve (Texasvets) of general veterinarians in Texas and we have begun the process of electronically mailing brochures to our referring veterinarians.

2d. Advertise clinical study via referring veterinarian seminars (months 6-18)

In August 2012 we arranged continuing education events with 4 veterinary medical associations in our referral area to promote the study; additionally, we plan to promote this trial at our annual continuing education conference in November 2012.

2e. Advertise clinical study via print media (months 6-18)

We hope to feature the study in our college magazine, CVM Today, in Fall 2012

2f. Development of standardized databases (months 8-10)

Databases for the study were developed between January and February 2012.

2g. Enrollment of dogs with IVDH (months 13-30)

It currently appears feasible to start enrollment at Month 15

Summary Task 2: It appears that we can begin enrollment as of study month 13. Pre-enrollment preparations are on schedule at TAMU.

Specific Aim 3

Task 1. Compare urodynamic measures in purpose bred dogs and dogs with IVDH (months 1-12)

1a. Obtain animal use protocol approval at Texas A&M University (months 1-12)

Obtained 8/12/2011

1b. Obtain Clinical Research Review Committee approval (months 1-4)

Completed 8/12/2011

1c. Order urodynamic equipment (month 1-4)

Urodynamic equipment was ordered in mid-December 2011 and arrived at TAMU in February 2011

1d. Order purpose bred dogs (month 4)

Purpose bred dogs were ordered in April 2012. As stated previously, this order was delayed due to delays in the production of GM 6001.

1e. Receive purpose bred dogs, allow for acclimatization (month 5)

Purpose bred dogs were obtained and acclimatized. The acclimatization process was completed in early May 2012.

1f. Perform urodynamic studies in purpose bred dogs (month 6). Ten purpose bred dogs will be utilized. Dogs will be sedated and will have a dual lumen urinary catheter, rectal catheter, and perineal

volume following micturition will be recorded and voided volume and voiding efficiency will be calculated. Baseline pressure (vesical pressure after voiding), maximal voiding pressure (maximal vesical pressure during micturition) or leak point pressure (maximal vesical pressure in an animal without voluntary voiding, prior to urine overflow), voiding duration, and voiding interval (the frequency of voiding during filling) will be determined. The number of uninhibited bladder contractions will be recorded on each study. Finally, the timing of external anal sphincter EMG activity in relation to the voiding will be examined. Dogs with phasic contractions of the external anal sphincter during voiding that exhibit subsequent interrupted urine flow and elevated voiding pressure will be classified as having reflex dyssynergia. Voided volume and voiding efficiency will be calculated. Bladder ultrasound will be performed in all dogs immediately following voiding on the same days as urodynamic studies to determine residual urine volume. Animals will be placed in cages and provided water for 8 hours. Upon removal from the cage, dogs will be walked in a large outdoor area and allowed to voluntarily void without manual assistance. Immediately following voiding, an ultrasound machine will be used to measure transverse depth, transverse width, longitudinal length, longitudinal depth, and longitudinal width of the bladder. These measurements will be utilized to calculate residual bladder volume as has been previously described in dogs with IVDH.

Ten healthy beagle-like dogs were utilized to generate experimental data in early May 2012. At the outset of this study, it became clear that Ketamine sedation would be inadequate as it produces excessive spasticity in dogs, which may interfere with the assessment of urodynamic measures. We modified our AUP and received ACURO approval to utilize dexmedetomidine as an alternative sedative agent.

1g. Perform urodynamic studies in dogs with IVDH (months 6-12). A total of 25 dogs not enrolled in the GM 6001 delivery trial will be utilized. Measurements will be performed at admission, and 3 days, 7 days, and 42 days following IVDH surgery. The same cystometric data as outlined in 1f will be recorded.

On June 1 2012 we opened enrollment to this clinical arm of the study. We have slightly modified inclusion criteria so that dogs lacking deep nociception are excluded due to the severity of the injury. Dogs lacking deep nociception have represented a small fraction (20%) of our IVDH associated SCI caseload and we did not believe that in a 25 dog population of dogs lacking deep nociception to make meaningful conclusions relative to typical urodynamic profile.

To date (8/24/12) we have enrolled 6 dogs; 5/6 dogs are still awaiting their 42 day re-check urodynamic profile due to their date of enrollment. While the study has only been running 12 weeks, we have completed 25% of the planned enrollment and plan to continue advertising to hopefully further facilitate the completion of this work in a timely manner. Additionally, will continue to enroll for this portion of the experiment during the completion of Specific Aim 3, Task 2 as study eligibility criteria a slightly different between these populations.

1h. Dr. Fosgate will analyze data (month 12). Descriptive statistics will be calculated for all urodynamic outcome measures in dogs. Evaluation of descriptive statistics and the Anderson-Darling test will be used to assess the normality assumption. The coefficient of variation will be calculated for the 3 replicates within unaffected dogs to assess the repeatability of the urodynamic measures. Urodynamic measures will be compared between normal and non-trail IVDH dogs at presentation using Student t tests for normally distributed variables and Mann-Whitney U tests otherwise. Outcome measures will be ranked based on the ability to distinguish normal versus affected dogs using scatter plots of standardized values and P values from the statistical comparisons. The outcome measure that best distinguishes affected from normal dogs will be used for subsequent statistical analyses. The most efficient urodynamic measure will also be compared between normal and IVDH-affected dogs at the 42 day recheck evaluation. Repeated measures ANOVA will be used to identify factors associated with improvement of urodynamic measures over time within the IVDH-affected dogs. Predictors that will be evaluated include time from injury until surgery, severity of injury at presentation, surgery duration, and age. Analyses will be performed in commercially available programs and results will be interpreted at the 5% level of significance.

Summary Specific Aim 3, Task 1: Cystometric measures and post-voiding bladder ultrasound was obtained in 10 Beagle-like dogs with few complications. In 2/10 dogs, hematuria was present following cystometry, but resolved within 24 hours. No dogs developed significant systemic complications as a result of cystometry.

To date, 6 dogs with IVDH-associated SCI have been enrolled in the serial cystometric study. At the time of cystometry, all dogs have been non-ambulatory with 3/6 being paraplegic. Thus, we appear to be obtaining a reasonable balance of mild and moderate SCI dogs. To date, at the time of injury dogs have had absent voiding reflex (5/6), residual volumes that are higher than control dogs, and baseline pressure that is higher than normal. Un-inhibited bladder contractions were seen in 1/6 dogs. Additionally, at the time of SCI bladder capacity appears larger than normal size matched animals. In all dogs, voiding reflexes have been seen by 7 day follow-up cystometry; this has correlated to complete or near-complete bladder emptying recognized on post-voiding ultrasound.

Figures 2 and 3 are cystometrograms obtained at the time of admission and 3 days following admission in one dog that demonstrate patterns seen during injury and recovery. At admission, there was an absence of voiding reflex, leak point pressure of 12 cm H2O, 75 mL of residual urine volume, and evidence of un-inhibited bladder contractions (Marked "U"). At 3 days following injury there was an absence of un-inhibited bladder contractions, a maximal voiding pressure of 40 cm H2O, voided volume of 78 mL with 28 mL of residual urine, and a baseline pressure following voiding of 8 cm H2O.

Task 2: Compare urodynamic measures in dogs with IVDH enrolled in the GM 6001 delivery trial (months 13-30).

Enrollment for this arm of the study is planned for month 15.

KEY RESEARCH ACCOMPLISHMENTS

UCSF Site:

- Developed reproducible models of graded spinal cord injuries in the mouse.
- Defined key parameters to assess urologic status in mice after spinal cord injury
- Conducted the first study to assess efficacy of GM6001 when delivered 8 hours after a moderate spinal cord injury in mice. Though group sizes were small, these data show promising results in terms of improving neurologic and urologic function.

TAMU Site:

- Completed pharmacokinetic study of GM 6001 in 10 dogs. The study supports the rapid development of maximal plasma concentration after S.Q. delivery, the presence of plasma drug levels capable of inhibiting MMPs in vitro, and the short term safety of the drug. Plasma drug levels following a single dose are sustained at a significant level likely to result in MMP inhibition for approximately 72 hours, which support a single dose strategy in the clinical trial.
- Completed normal dog cystometry in 10 dogs
- Enrolled 6 dogs with acute IVDH-associated SCI into serial cystometry study. As expected, dogs with SCI that are non-ambulatory lack normal voiding reflex, have larger bladder capacity, have elevated post-cystometry baseline pressure, and have larger residual volume compared to measures taken during recovery.

REPORTABLE OUTCOMES

UCSF Site:

Poster presentation at the MHSRS/ATACC meeting, August 13-16, 2012 in Fort Lauderdale. Abstract was entitled “Urinary bladder dysfunction in a murine model of spinal cord injury: Relationship between injury severity and measures of urologic status”. Abstract is provided in the **Appendices.**

TAMU Site:

Our group has pending grants totaling $475,000 to Mission Connect Foundation, Siemens Corp, and Neilsen Foundation since data collection for this DoD sponsored project began. These proposed studies include serial high resolution MRI of the spinal cord and correlates to histopathology as well as a whole exome sequencing project to detect genomic variants responsible for SCI severity. While data from the DoD study were not directly used, we have highlighted that the ease and rate of enrollment supports the feasibility of projects such as those currently proposed.

CONCLUSIONS

- In a preliminary study, GM6001 when given 8 hours after a moderate spinal cord injury in the mouse, results in improvement in long-term neurologic recovery and a significant reduction in abnormal bladder contractility.
- GM 6001 dosed subcutaneously at 100 mg/kg in dogs is safe and results in a pharmacokinetic profile that lends itself to the duration of MMP inhibition demonstrated to be effective in rodent neurotrauma work
- In dogs with IVDH associated SCI, urinary voiding impairment can be assessed by cystometry and bears similarity to what is seen in humans with per-acute injury. Voiding recovery happens rapidly in dogs with mild or moderate SCI (non-ambulatory with or without limb movement but with intact deep nociception).

REFERENCES

1 Noble LJ et al *Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. Journal of Neuroscience 22, 7526 (2002)*

APPENDICES:

Poster presentation at the MHSRS/ATACC meeting, August 13-16, 2012 in Fort Lauderdale.

TITLE: Urinary bladder dysfunction in a murine model of spinal cord injury: Relationship between injury severity and measures of urologic status

Thomas M. Fandel, Jonathan Levine, Kayleen Gimlin, Haoqian Zhang,
Alpa Mahuvakar, and Linda J. Noble-Haeusslein
Presenter's Name: Linda J. Noble-Haeusslein, Ph.D.

PURPOSE/AIMS: The purpose of this study was to determine the extent to which severity of an incomplete spinal cord injury (SCI) influences bladder function in a murine model of SCI.
DESIGN: Mice were randomized to sham, (n=8), mild (n=5) or moderate (n=7) SCI and treated with Enrofloxacin for 10 days subcutaneously followed by food supplemented with Enrofloxacin until euthanasia. Neurological status was evaluated at 1 and 3 days post injury and weekly thereafter for 3 weeks. At 4 weeks post-injury, awake cystometry was performed (n= 3-7/group). At the completion of cystometry and after residual urine was determined (n=3-6/group) the bladders were removed and weighed (n= 5-7/group). All observers were blinded to the experimental condition.
POPULATION/SAMPLE STUDIED: Adult, male, C57Bl6 mice subjected to laminectomy only or mild or moderate SCI.
METHODS: SCI was produced by dropping either a 2 g (mild injury) or 3 g (moderate injury) weight onto the exposed spinal cord at the T9 vertebral level. Neurological status was based upon the BMS scale. At 3 weeks post-injury, a PE50 polyethylene catheter was implanted into the bladder dome and tunneled subcutaneously to emerge in the interscapular area. One week later, cystometry was performed in the awake restrained animal using saline at an infusion speed of 16-20 ml/ minute (Catamount Research, St. Albans, VT). Residual urine was determined at the end of cystometry. The urinary bladders were removed, blotted dry, and weighed.
DATA ANALYSIS: Two-way repeated measures (RM) analysis of variance (ANOVA) was used to evaluate neurological recovery. Residual urine and bladder weight were analyzed using 1-way ANOVA followed by Bonferroni's Multiple Comparison Test. Unpaired Student's T-test was used when two groups were specified. Significance was defined at $P \leq 0.05$. All data are expressed as means +/- SEM.
FINDINGS: BMS scores revealed an effect of both time (p=0.0001) and injury severity (p=0.0182). While both injury groups showed improved performance over time, BMS scores were lower in the 3 g (1.786$\pm$0.3595) relative to the 2 g (6.000$\pm$1.508) group (p=0.0097) at 21 days post injury. Moreover, a 3 g injury led to qualitatively more uninhibited bladder contractions and greater residual urine (0.9293$\pm$0.1346) and bladder weight (0.1475+/0.2238 g) relative to residual urine (0.3980$\pm$0.0080) and bladder weight in the 2 g injury (0.07160$\pm$0.0072 g) ($p<0.01$). CONCLUSIONS/RECOMMENDATIONS: There are injury severity dependent abnormal changes in both weight and function of the urinary bladder after SCI.
IMPLICATIONS: While bladder dysfunction is a common problem in human SCI, analyses of bladder function are typically neglected in murine models of SCI. Characterization of bladder function, relative to injury severity, provides a clinically relevant benchmark for establishing efficacy of candidate therapeutics.
FROM/TO TIME PERIOD OF STUDY: From September 30, 2011 to April 25, 2012
FUNDING: DOD Spinal Cord Injury Program SC100140

SUPPORTING DATA- TAMU

Figure 1: Plasma concentration of GM 6001 in 5 dogs dosed once at 100 mg/kg S.C. and 5 dogs dosed twice at a 12-hour interval. Data for the two-dose cohort was collected only at time points following the second dose.

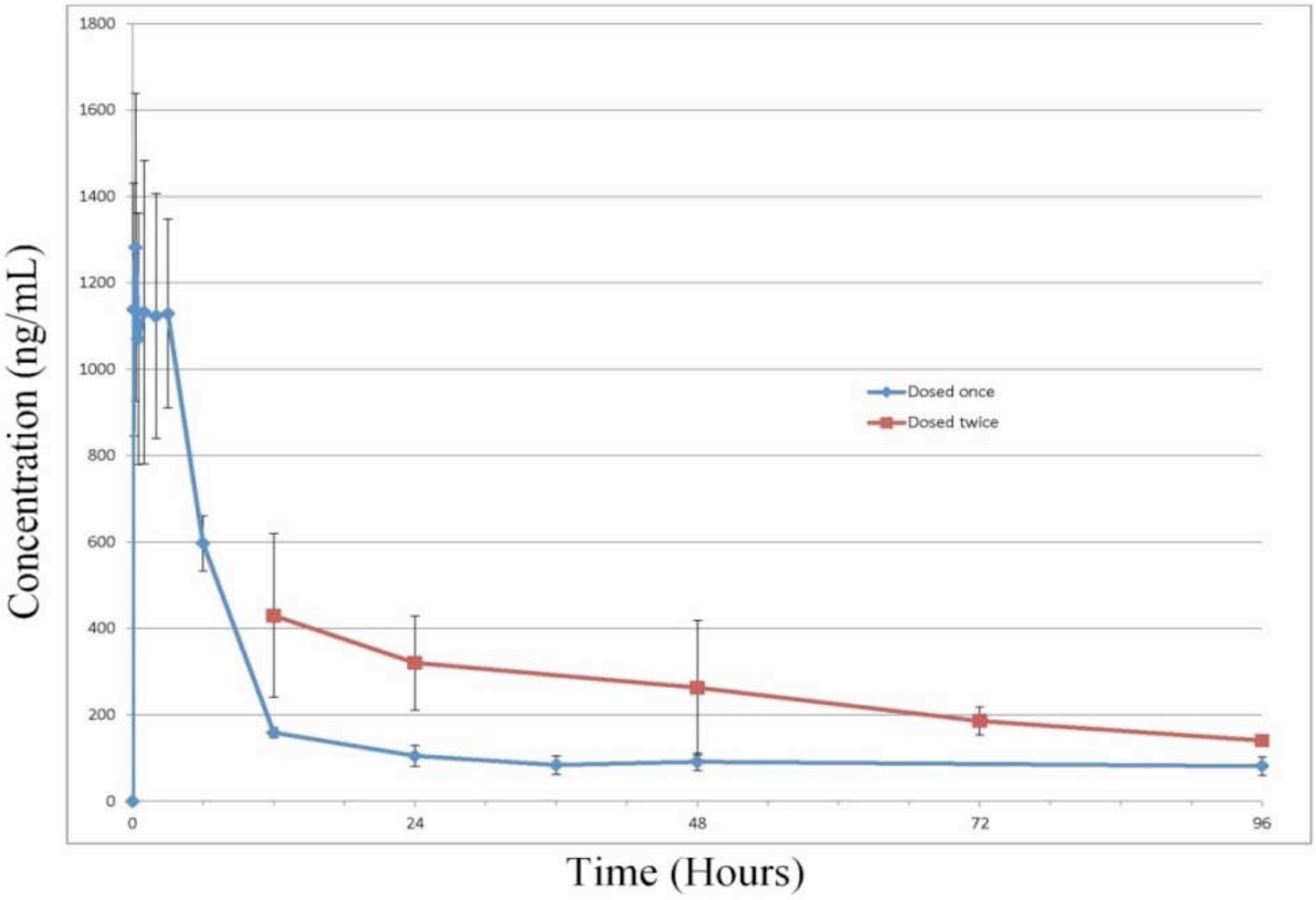


Figure 2. Cystometry 24 hours following spinal cord injury in a dog with intervertebral disk herniation. Note the absence of voiding, the presence of un-inhibited bladder contractions (U), and the low leak point pressure (12 mm H2O)

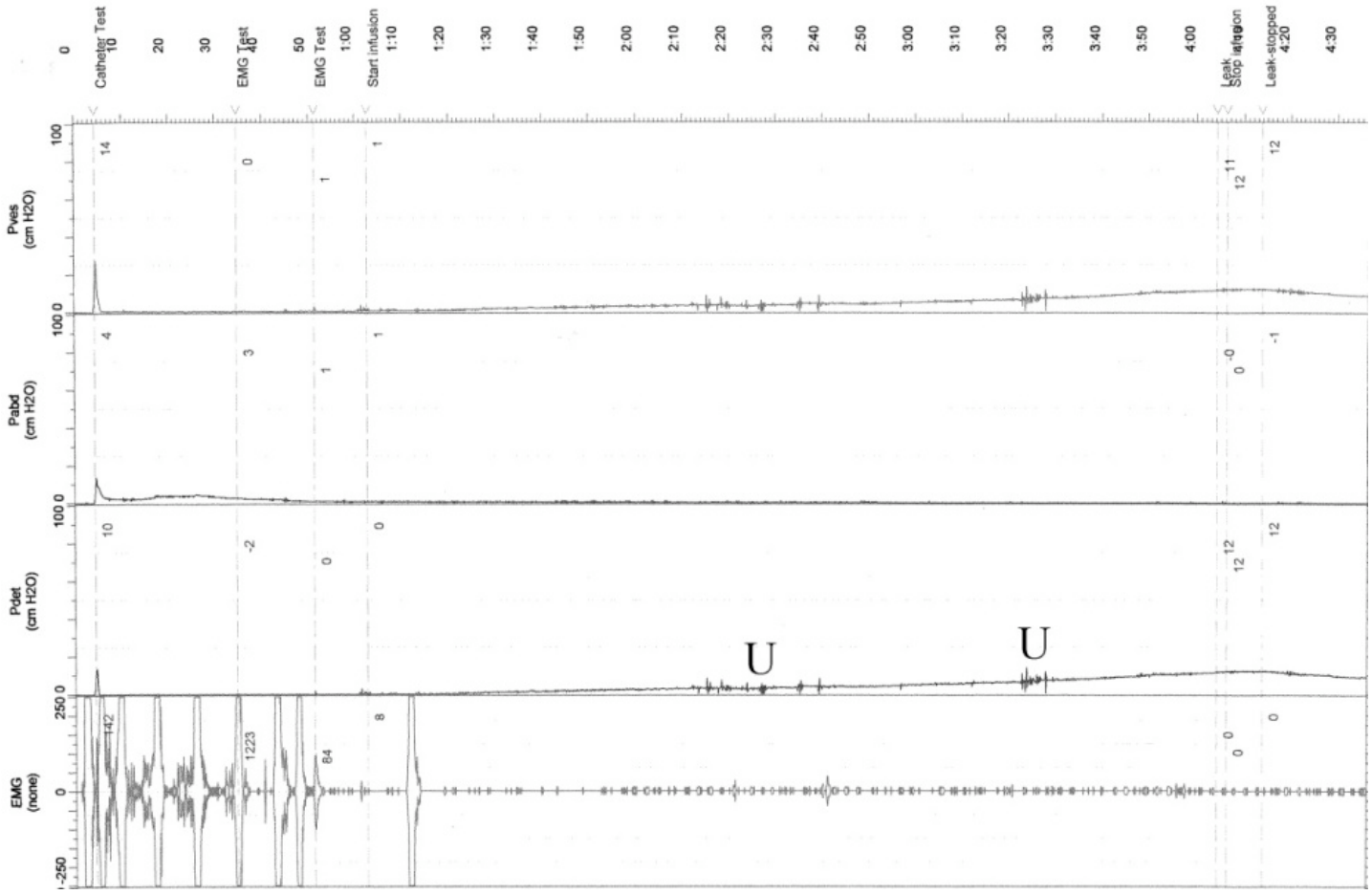


Figure 3. Cystometry 3 days following Figure 2. Note the presence of a voiding reflex, the absence of uninhibited bladder contractions, and the presence of anal EMG activity.